

Novel Sodium Channel Blockers**Related Application**

This application claims priority under 35 USC §199(e) to US Provisional
5 Application Serial Nos. 60/373,440, filed April 18, 2002, and 60/373,784, filed April 19,
2002, the disclosures of which are incorporated herein.

Field of the Invention

The present invention is directed to novel compounds and the use of such
10 compounds as sodium channel blockers. Such compositions have utility in treating diseases
associated with inappropriate sodium channel activity, and include the use of these
compounds as anti-cancer agents.

Background of the Invention

15 The capacity of a cell to alter its morphology and migrate is inherent to cancer
cell metastasis. Although the precise biological mechanisms shaping cellular morphology
during metastasis have not been elucidated, it is known that such changes involve cell-matrix
interactions and cytoskeletal elements. The involvement of Na⁺ channels in shaping cellular
morphology has been described for neurons (Mattei et al., Journal of Neuroscience Research,
20 55(6):666-73, 1999). As of yet, the intracellular mechanisms through which Na⁺ channel
activity regulate cancer cellular morphology are unclear, although ion channels have been
implicated in several types of cellular behavior that could be related to the different stages of
metastasis. These include proliferation, migration, and adhesion/interaction with the cellular
matrix.

25 Voltage-gated ion channels, classically associated with impulse conduction in
excitable tissues, are also found in a variety of epithelial cell types where their function is not
well known. Nine mammalian sodium channel genes have been identified and found to be
expressed and functional. These genes are greater than 50% identical in amino acid sequence
in the transmembrane and extracellular domains. Recently, several types of voltage-gated ion
30 channels have been discovered in rat and human prostate cancer cells. Several independent
studies have also linked a prostate voltage gated sodium (Na⁺) channel α -subunit with the
invasiveness of human prostate cell lines including LNCaP and PC-3 (see Diss, et al., The
Prostate, 48:165-178, 2001 and Smith et al., FEBS Letters, 423:19-24, 1998.). Further,
electrophysiological studies using a whole-cell patch clamp indicated that the identified

prostate cell sodium channel is sensitive to tetrodotoxin (TTX) at 600 nM, identifying the channel as voltage dependent TTX sensitive Na⁺ channel protein.

Comparisons between rodent and human prostate cancer cell lines led to the conclusion that the level of Na⁺ channel expression is associated positively with the invasiveness of prostate cancer cells *in vitro*. Encouragingly, both protein and functional studies strongly support sodium channel blockade as a viable mechanism for PCa cell inhibition. Recently, the effect of four anticonvulsants on the secretion of prostate-specific antigen (PSA) and interleukin-6 (IL-6) by human prostate cancer cell lines (LNCaP, DU-145 and PC-3) was measured using ELISA's specific for each protein. The results demonstrated that both phenytoin and carbamazepine, which inactivate voltage-gated sodium channels (NVSC), inhibit the secretion of PSA by LNCaP and IL-6, DU-145 and PC-3 cell lines (Abdul, M. and Hoosein, N., *Anticancer Research*, 21(3B):2045-8, 2001 May-Jun). Additionally, the authors demonstrate a reduced capacity to form colonies in Matrigel upon treatment with phenytoin. These data indicate further that sodium channel blockade is a strong candidate for effective treatment of PCa.

Experiments using tritiated batrachotoxin (BTX) have revealed an allosteric relationship between BTX and the phenytoin binding site in brain tissue. This relationship led applicant to investigate the neuronal hydantoin receptor in the brain for conformation and lipophilic properties. Since there was little structural data about the phenytoin-binding site on the NVSC, a defined series of compounds was designed, synthesized and evaluated to identify novel Na⁺ channel blocking agents. Such compounds have utility in treating diseases associated with hyper sodium channel activity, including treating epilepsy, pain, bipolar disease, depression Amyotrophic lateral sclerosis (ALS) and neoplastic disease such as androgen-sensitive and androgen-independent prostate cancer.

Prostate neoplasia is the most common cause of cancer in men and the second leading cause of cancer death among men in the U.S. Approximately 189,000 men will be diagnosed with prostate cancer and approximately 30,000 will die from this disease in 2002. Human prostate cancer cells express a voltage gated sodium channel, a 260 Kd transmembrane protein that is similar to neuronal subtypes. Whole cell patch clamping experiments indicate that the prostate voltage sodium channel (PVSC) also functions similarly to neuronal subtypes. Significantly, Na⁺ Channel expression in prostate cancer cells has been correlated positively to invasiveness in the highly metastatic cell line MAT-LyLu (rat). PVSC has been found to be sensitive to Tetrodotoxin (TTX) and it has been reported that TTX inhibits the invasiveness of PC-3 cells (human) by 31% (P=0.02)

Laniado, et al. American Journal of Pathology, 150(4): 1213-21, 1997. Furthermore, TTX (6 mM) produces alterations in prostate cancer cell morphology, including a decrease in cell process length, field diameter; increases in cell body diameter and process thickness. S.P. Fraser, Y. Ding, A. Liu, C.S. Foster M.B.A. Djamgoz. Cell Tissue Research. 295: 505-512, 1999 and Grimes JA. Djamgoz MB. Journal of Cellular Physiology. 175(1):50-8, 1998. Therefore, PVSC serves as an effective target for potential prostate cancer therapeutics, thus presenting a need for new inhibitors of this sodium channel.

Summary of the Invention

The present invention is directed to the design and synthesis of novel voltage-gated sodium channel (VGSC) and prostate voltage sodium channel (PVSC) inhibitors. Compositions comprising such inhibitors have utility in treating diseases characterized by overabundant or hyperactive VGSC/ PVSC's. In one embodiment a sodium channel binder/blocker that selectively targets overabundant or hyperactive VGSC's in the prostate is used to limit or prevent PCa growth and/or metastasis.

Brief Description of the Drawings

Fig. 1: Effects of phenytoin (DHP) and analogues 1, 5 and 44 on the rNav1.2. Currents were elicited by a step depolarisation from a holding potential of -100mV to +10mV for 50 msec at 15 sec intervals. In each example a control trace is superimposed with one recorded at maximum drug affect. All compounds were tested at 100µM concentration.

Detailed Description of the Invention

Definitions

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, the term "purified" and like terms relate to the isolation of a molecule or compound in a form that is substantially free (at least 60% free, preferably 75% free, and most preferably 90% free) from other components normally associated with the molecule or compound in a native environment.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein, an "effective amount" means an amount sufficient to produce a selected effect. For example, an effective amount of a sodium channel blocker is an amount of the blocker sufficient to produce a detectable inhibition of sodium channel activity.

The general chemical terms used in the description of the compounds of the present invention have their usual meanings. For example, the term "alkyl" by itself or as part of another substituent means a straight or branched aliphatic chain having the stated number of carbon atoms.

The term "halo" includes bromo, chloro, fluoro, and iodo.

The term "haloalkyl" as used herein refers to an alkyl radical bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term " C_1 - C_n alkyl" wherein n is an integer, as used herein, refers to a branched or linear alkyl group having from one to the specified number of carbon atoms. Typically C_1 - C_6 alkyl groups include, but are not limited to, methyl, ethyl, *n*-propyl, isopropyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

The term " C_2 - C_n alkenyl" wherein n is an integer, as used herein, represents an olefinically unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one double bond. Examples of such groups include, but are not limited to, 1-propenyl, 2-propenyl, 1,3-butadienyl, 1-butenyl, hexenyl, pentenyl, and the like.

The term " C_2 - C_n alkynyl" wherein n is an integer refers to an unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one triple bond. Examples of such groups include, but are not limited to, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 1-pentyne, and the like.

As used herein, the term "optionally substituted" refers to zero to four substituents, wherein the substituents are each independently selected. More preferably, the term refers to zero to three independently selected substituents.

As used herein the term "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like. Aryl groups (including bicyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from lower alkyl, haloalkyl, alkoxy, amino, alkylamino, dialkylamino, hydroxy,

halo, and nitro. The term (alkyl)aryl refers to any aryl group which is attached to the parent moiety via the alkyl group.

The term "C₃-C_n cycloalkyl" wherein n = 4-8, represents cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl.

5 The term "heterocyclic group" refers to a C₃-C₈ cycloalkyl group containing from one to three heteroatoms wherein the heteroatoms are selected from the group consisting of oxygen, sulfur, and nitrogen.

The term "bicyclic" represents either an unsaturated or saturated stable 7- to 12-membered bridged or fused bicyclic carbon ring. The bicyclic ring may be attached at any
10 carbon atom which affords a stable structure. The term includes, but is not limited to, naphthyl, dicyclohexyl, dicyclohexenyl, and the like.

The term "lower alkyl" as used herein refers to branched or straight chain alkyl groups comprising one to eight carbon atoms, including methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, neopentyl and the like.

15 The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous.

As used herein, the term "treating" includes alleviating the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms.

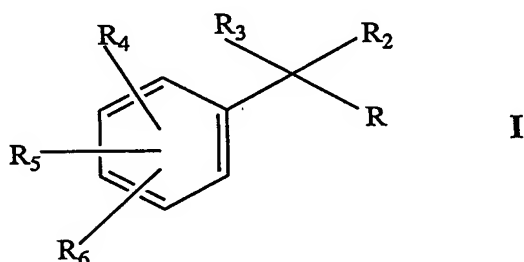
20 As used herein the term "anti-tumor agent" relates to agents known in the art that have been demonstrated to have utility for treating neoplastic disease. For example, antitumor agents include, but are not limited to, antibodies, toxins, chemotherapeutics, enzymes, cytokines, radionuclides, photodynamic agents, and angiogenesis inhibitors. Toxins include ricin A chain, mutant Pseudomonas exotoxins, diphtheria toxoid, streptonigrin, boamycin, saporin, gelonin, and pokeweed antiviral protein.
25 Chemotherapeutics include 5-fluorouracil (5-FU), daunorubicin, cisplatin, bleomycin, melphalan, taxol, tamoxifen, mitomycin-C, and methotrexate. Radionuclides include radiometals. Photodynamic agents include porphyrins and their derivatives. Angiogenesis inhibitors are known in the art and include natural and synthetic biomolecules such as
30 paclitaxel, O-(chloroacetyl-carbonyl) fumagillol ("TNP-470" or "AGM 1470"), thrombospondin-1, thrombospondin-2, angiostatin, human chondrocyte-derived inhibitor of angiogenesis ("hCHIAMP"), cartilage-derived angiogenic inhibitor, platelet factor-4, gro-beta, human interferon-inducible protein 10 ("IP10"), interleukin 12, Ro 318220, tricyclodecan-9-yl xanthate ("D609"), irsogladine, 8,9- dihydroxy-7-methyl-

benzo[b]quinolizinium bromide ("GPA 1734"), medroxyprogesterone, a combination of heparin and cortisone, glucosidase inhibitors, genistein, thalidomide, diamino-antraquinone, herbimycin, ursolic acid, and oleanolic acid.

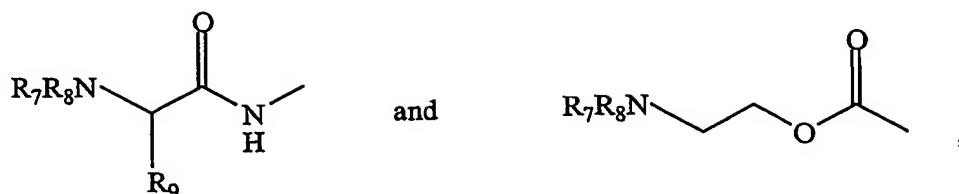
The novel VGSC blockers of the present invention contain one or more asymmetric centers in the molecule. In accordance with the present invention a structure that does not designate the stereochemistry is to be understood as embracing all the various optical isomers, as well as racemic mixtures thereof.

The Invention

The present invention relates to the discovery of novel sodium channel blockers and the use of those compounds to treat diseases associated with excessive voltage gated sodium channel activity. In accordance with the present invention a modulator of voltage gated sodium channels is provided wherein the modulator has the general structure:

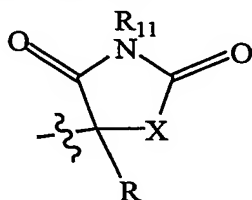


wherein R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, -(CH₂)_mCOOH, -(CH₂)_mNH₂, -(CH₂)_mCONH₂, -(CH₂)_nC₃-C₆ cycloalkyl, -(CH₂)_p(CHOH)CONH₂, -(CH₂)_nsubstituted aryl, -(CH₂)_pNCH₃(CH₂)_psubstituted aryl, -(CH₂)_naryl, and -(CH₂)_nsubstituted heterocyclic, wherein m is an integer ranging from 3-8, n is an integer ranging from 0-4 and p is an integer ranging from 1-4. R₂ is selected from the group consisting of H, C₁-C₈ alkyl, -(CH₂)_nCOOH, -(CH₂)_nNH₂, -(CH₂)_nNHCH₃, and -(CH₂)_nCONHR₁₀, R₃ is selected from the group consisting of H, hydroxy, amino, (C₁-C₄) alkoxy, -CH₂OH and -CONH₂, or R₂ and R₃ taken together with the atoms to which they are attached form an optionally substituted aryl or an optionally substituted heterocyclic ring, R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl, -COR₁₁ and (C₁-C₄) alkoxy, and R₆ is selected from the group consisting of H, halo, C₁-C₈ alkyl, amino, hydroxy, C₁-C₈ alkoxy,



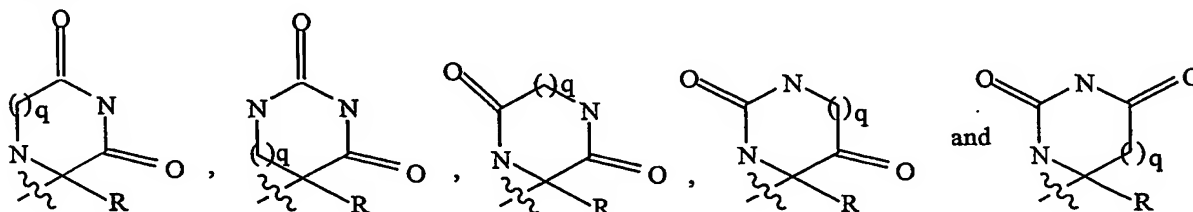
wherein R_7 and R_8 are independently selected from the group consisting of H, C_1 - C_4 alkyl, C_2 - C_4 alkenyl and C_2 - C_4 alkynyl, and R_9 is H, or R_8 and R_9 taken together with the atoms to which they are attached form an optionally substituted heterocyclic ring, R_{10} is selected from the group consisting of H and C_1 - C_4 alkyl, and R_{11} is selected from the group consisting of H, C_1 - C_4 alkyl, NH_2 and OH , with the proviso that when R_4 , R_5 and R_6 are each H, and R_2 and R_3 taken together form a heterocyclic ring, R is not $-(CH_2)_n$ aryl.

In one embodiment the compound has the general structure of Formula I, wherein R_2 and R_3 taken together with the atoms to which they are attached form a heterocyclic ring having the structure:



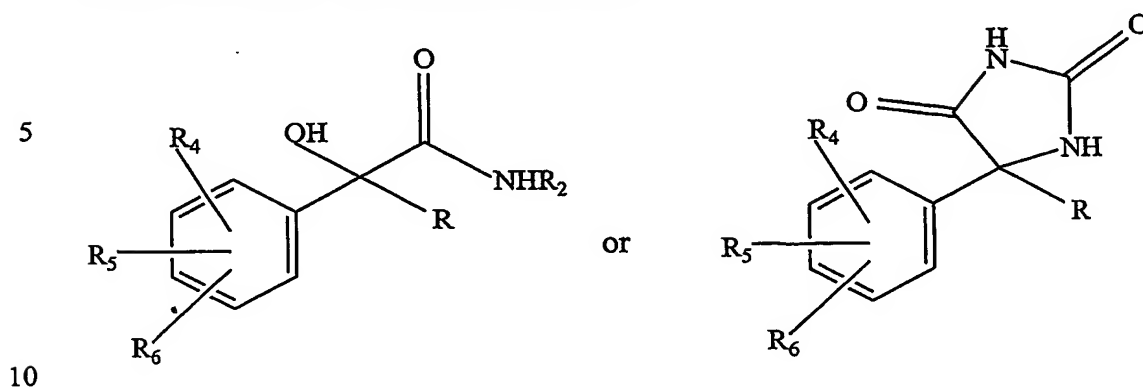
wherein X is selected from the group consisting of $-CHR_{12}-$, $-O-$ and $-NR_{12}-$, wherein R_{11} and R_{12} are independently selected from the group consisting of H, benzyl and C_1 - C_4 alkyl, with the proviso that when X is $-NH_2-$ and R_{11} is H, R is not phenyl. In one preferred embodiment X is $-NR_{12}-$.

In one embodiment the compound has the general structure of Formula I, wherein R is selected from the group consisting of C_1 - C_{12} alkyl, C_2 - C_8 alkenyl, C_2 - C_8 alkynyl, R_2 and R_3 taken together with the atoms to which they are attached form a ring having the structure:

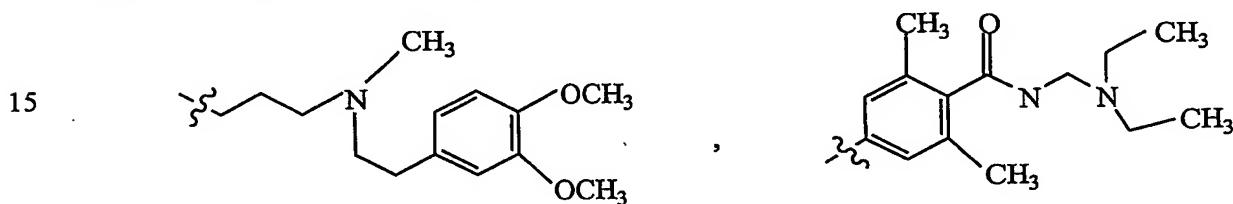


wherein q is an integer ranging from 1 to 2. In one preferred embodiment R is C_1 - C_{12} alkyl, q is 1 and R_4 , R_5 and R_6 are independently selected from the group consisting of H and halo.

In accordance with one embodiment a sodium channel blockers is provided wherein the blocker has the general structure:

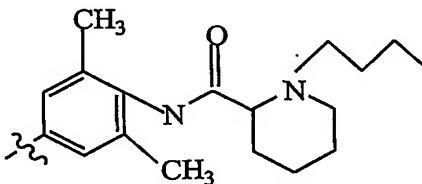


wherein R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, -(CH₂)_nC₃-C₆ cycloalkyl,



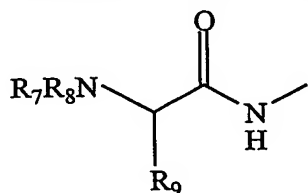
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and

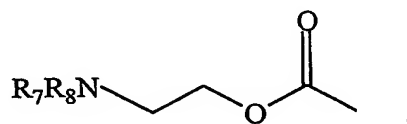


wherein n is an integer ranging from 0-4. R₂ is H or C₁-C₄ alkyl, R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl, -COR₁₀ and (C₁-C₄) alkoxy, and R₆ is selected from the group consisting of H, halo, C₁-C₈ alkyl, amino, hydroxy, C₁-C₈ alkoxy and

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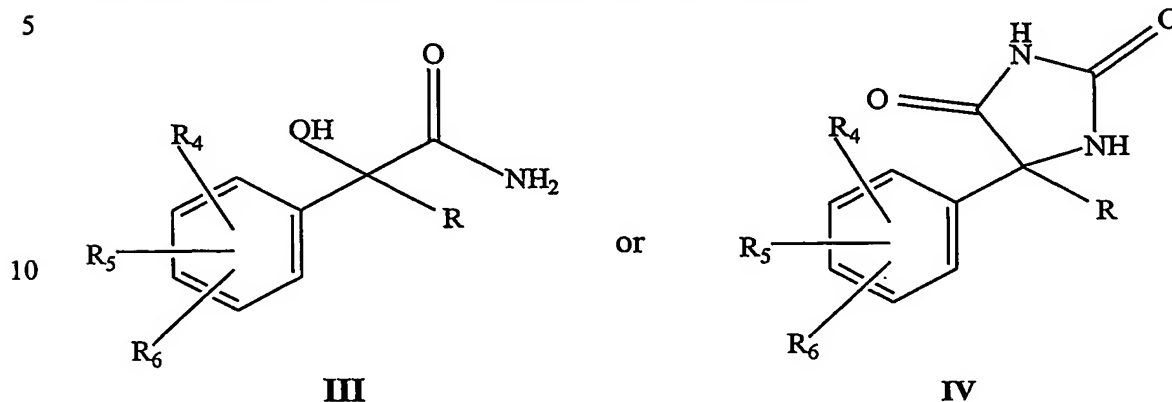
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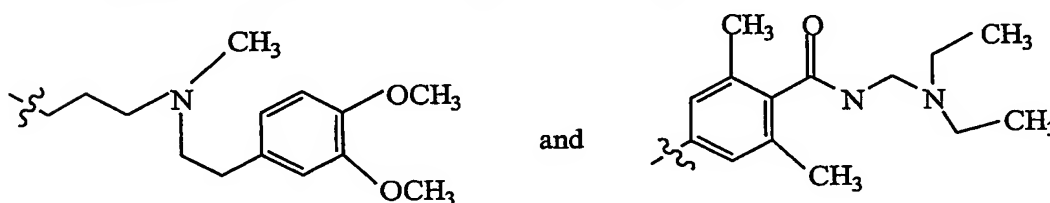
wherein R₇ and R₈ are independently selected from the group consisting of H, C₁-C₄ alkyl, C₂-C₄ alkenyl and C₂-C₄ alkynyl, and R₉ is H, or R₈ and R₉ taken together with the atoms to

which they are attached form an optionally substituted heterocyclic ring and R₁₀ is selected from the group consisting of H, C₁-C₄ alkyl, NH₂ and OH.

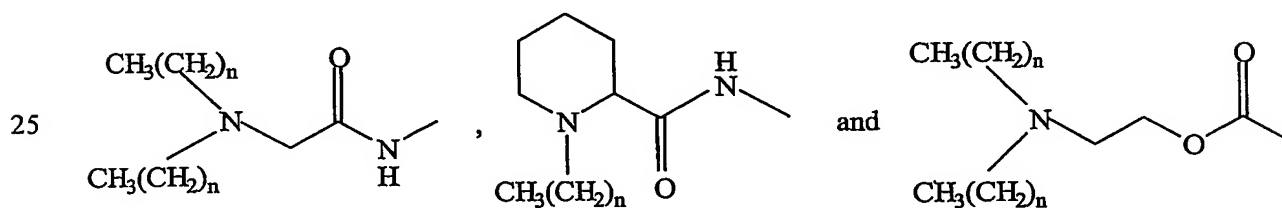
In one embodiment a compound that modulates sodium channel activity is provided wherein the compound is represented by the general structure



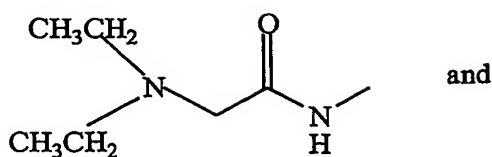
wherein R is selected from the group consisting of C₃-C₁₀ alkyl,



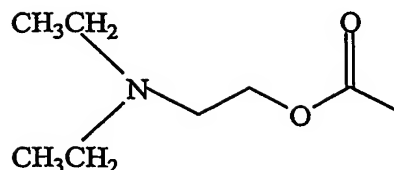
R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, and -OCH₃, and R₆ is selected from the group consisting of H,



wherein n is an integer ranging from 0-4. In accordance with one embodiment the compound has the general structure of Formula III or IV wherein R is C₃-C₉ alkyl and R₄ and R₅ are independently H or methyl and R₆ is selected from the group consisting of

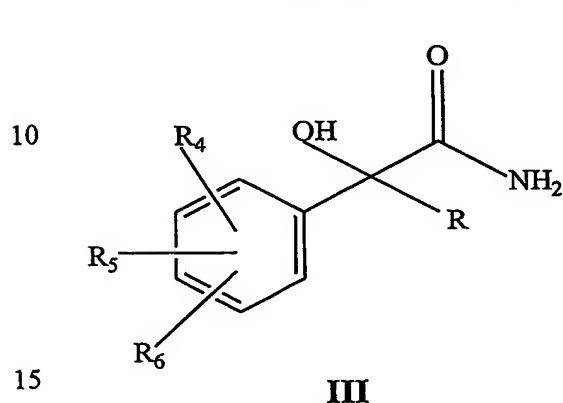


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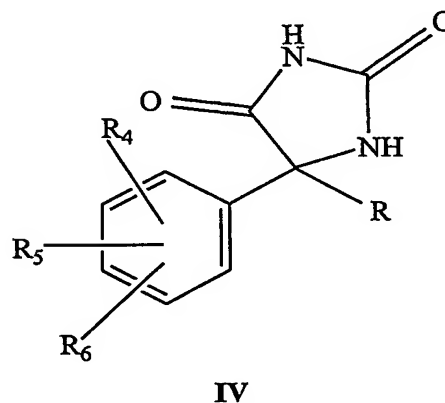


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In accordance with one embodiment of the present invention a compound is provided that is represented by the general structure



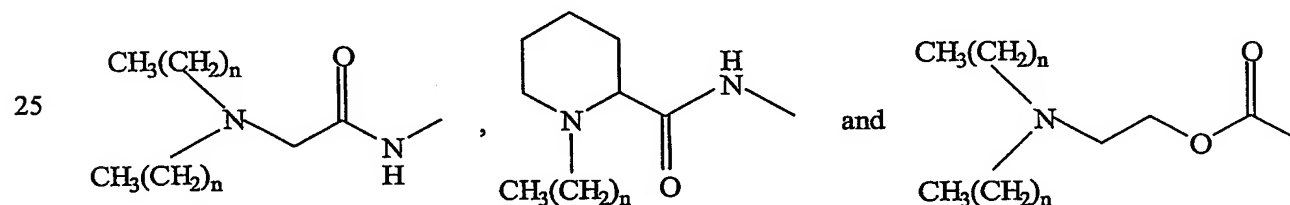
or



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wherein R is selected from the group consisting of C₃-C₉ alkyl, C₂-C₉ alkenyl, and C₂-C₉ alkynyl,

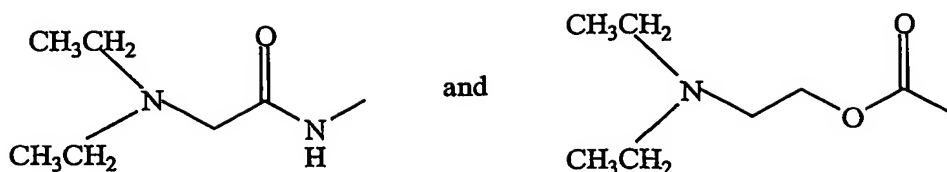
R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl, and -OCH₃, and R₆ is selected from the group consisting of H, halo, C₁-C₈ alkyl, amino, hydroxy, and



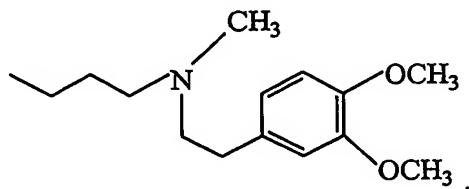
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wherein n is an integer ranging from 0-4. In accordance with one embodiment the compound has the general structure of Formula III or IV wherein R is C₃-C₁₂ alkyl and R₄, R₅ and R₆ are independently selected from the group consisting of H and halo. In an alternative embodiment, the compound has the general structure of Formula III or IV wherein R is C₃-C₁₂ alkyl, R₄ and R₅ are independently H or C₁-C₄ alkyl and R₆ is selected from the group consisting of

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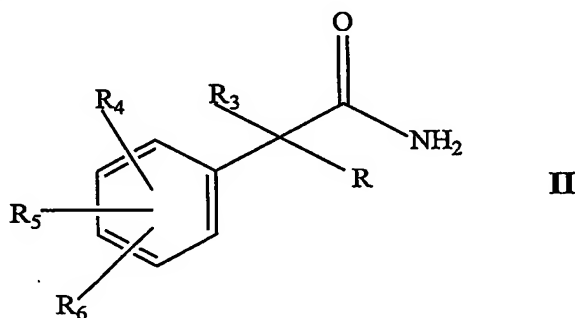


In another embodiment of the present invention a compound of the general formula III or IV is provided wherein R is

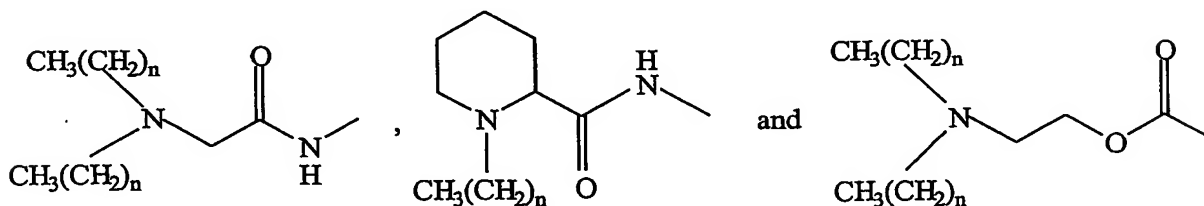


R_4 and R_5 are independently selected from the group consisting of H, halo, C_1 - C_4 alkyl, and $-OCH_3$, and R_6 is H.

In another embodiment the sodium channel blocker of the present invention has the general structure



wherein R is selected from the group consisting of C_1 - C_{12} alkyl, C_2 - C_9 alkenyl and C_2 - C_9 alkynyl, R_3 is hydroxy, amino, (C_1 - C_4) alkoxy, $-CH_2OH$ or $-CONH_2$, R_4 and R_5 are independently selected from the group consisting of H and halo, and R_6 is selected from the group consisting of H, halo and



wherein n is an integer ranging from 0-4, and in one embodiment n is 1 or 2. In one preferred embodiment, a sodium channel blocker represented by formula II is provided wherein R is

C₁-C₁₂ alkyl, R₃ is hydroxyl, R₄ is halo and R₅ and R₆ are halo or H, and in one preferred embodiment the halo substituents are either F or Cl. In a further embodiment a compound of the general structure of Formula II is provided wherein R is C₅-C₉, R₃ is hydroxyl, R₄ and R₅ are both H and R₆ is halo, and more preferably F or Cl. In one preferred embodiment the present invention is directed to compounds of the general structure of Formula II wherein R is -(CH₂)₆CH₃, R₃ is hydroxyl, R₄ and R₅ are both H and R₆ is para-F or meta-Cl.

One aspect of the present invention is directed to the inhibition of voltage gated sodium channels as a novel method of targeting neoplastic cells and inhibiting metastasis. Several studies have identified the presence of sodium channel isotypes in prostate cancer cells. Sodium channel mRNA from two highly metastatic prostatic epithelial tumor cell lines MAT-Ly-Lu (rat) and PC-3 (human) was identified as the full-length skeletal muscle type 1 (SkM1). *In situ* hybridization data suggests that the level and pattern of rSkM1 mRNA expression were different in the Dunning cells of markedly different metastatic potential. Interestingly, the same type of mRNA was also detected in the weakly metastatic counterparts of AT-2 (rat) and LNCaP (human) PCa cells. Diss et. al. (The Prostate, 48:165-178, 2001), used semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) to determine the expression profile of sodium channel mRNAs in several prostate cancer (PCa) cell lines. These results indicate that four different VGSC genes with 9 splice variants are expressed in LNCaP cells while PC-3 has 11 splice variants of the same mRNAs. Many of these splice variants encode the fetal form of the VGSC. It has recently been reported that prostate cancer cells express a voltage gated sodium channel (VGSC) and that the activity of this channel protein correlates with cellular invasiveness.

A study of VGSCs in the LNCaP and PC-3 human prostate cancer cell lines by Western blotting and flow cytometry reveal this channel to be a 260-kd protein representative of an alpha subunit (Komuro, et al., Science, 257: 806-809, 1992). Electrophysiological studies, using the whole-cell patch clamp technique demonstrate that the current elicited by this channel was inhibited by tetrodotoxin (TTX) at 600 nmol/L, thus identifying the subunit as a Na⁺ channel. Furthermore, it has been reported that the highly metastatic (rat) MAT-LyLu PCa cell line, derived from the Dunning model of rat prostate cancer, express a VGSC while the less metastatic (rat) AT-2 PCa cell line does not. Blockage of Na⁺ current with TTX significantly reduced the invasiveness of the MAT-LyLu cells *in vitro*, suggesting that the expressed channel has a functional role in metastasis. Moreover, the invasiveness of MAT-LyLu cells *in vitro* was inhibited by up to 50% with 6 μM TTX, a specific VGSC inhibitor. TTX exposure (incubation of MAT-LyLu for 24 h with 6 μM TTX) also altered

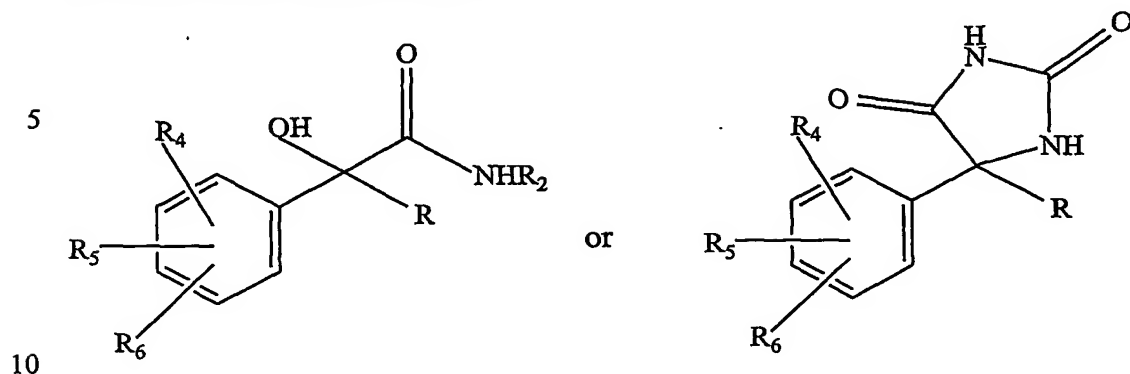
several morphological features associated with an aggressive or highly motile phenotype, and more particularly, exposure to TTX decreased cell process length, field diameter, and increasing cell body diameter and process thickness.

5 The results obtained using TTX suggest that the Na⁺ channel may play a significant role in determining the morphological development of MAT-Ly-Lu cells in such a way as to enhance their metastatic potential. Further characterization of the current of these channels, using the whole-cell patch clamping, was conducted and the measured currents were compared to Na⁺ currents found in various other tissues. It was shown that the inward current of the Mat-Ly-Lu cells was abolished completely, but reversibly, in Na⁺ -free
10 solution. This confirms that Na⁺ was indeed the permeant ion. Similar data were obtained from human PCa cell lines. PC-3 cells treated with TTX had a 31% (P = 0.02) reduction of invasiveness *in vitro* using Boyden chamber assays. The TTX mediated reduction in the invasiveness of PC-3 cells strongly suggests that ion channel modulators play an important functional role in human tumor invasion. However, TTX (which comes from puffer fish) is
15 very toxic to live organisms and thus is not a suitable candidate for pharmaceutical formulations.

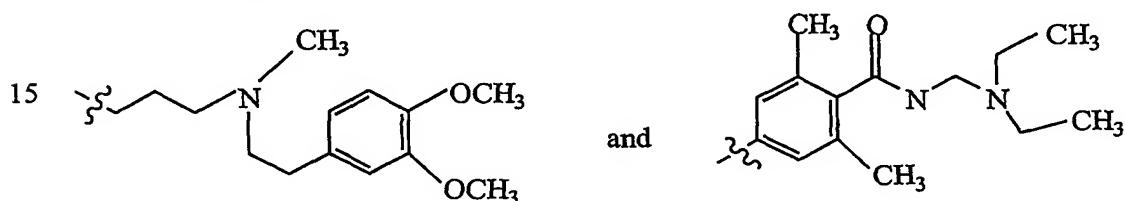
Recently, Abdul et. al., demonstrated that PCa specimens have higher levels of sodium channel expression compared to normal prostate (see Anticancer Research, 21(3B):2045-8, 2001). In addition, they also showed that a VGSC-opener (veratrine)
20 increased proliferation, while VGSC-blockers (flunarizine and riluzole) caused dose dependent inhibition of PCa cell growth in the micromolar range. Taken together, these studies establish that PCa cell lines from both rat and human express VGSCs that are part of the tumorigenic behavior of these cell lines.

In accordance with one embodiment of the present invention a method is
25 provided for treating a warm blooded vertebrate patient, including humans, afflicted by a neoplastic disease, such as prostate cancer. The method comprises the steps of administering

to such a patient an effective amount of a composition comprising a sodium channel blocker represented by the general structure:



wherein R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, -(CH₂)_nC₃-C₆ cycloalkyl,

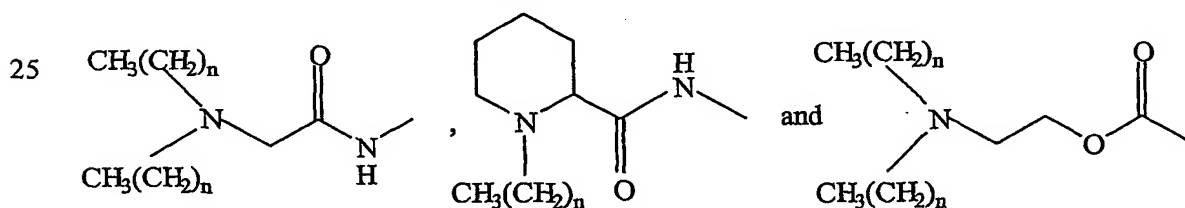


wherein n is an integer ranging from 0-4;

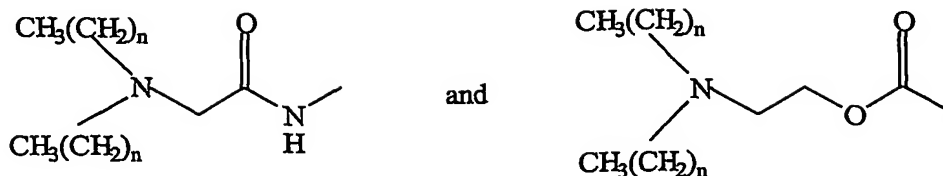
R₂ is selected from the group consisting of H, C₁-C₈ alkyl, -(CH₂)_nCOOH, -
20 (CH₂)_nNH₂, -(CH₂)_nNHCH₃, and -(CH₂)_nCONH₂;

R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl, -COR₁₁ and (C₁-C₄) alkoxy; and

R₆ is selected from the group consisting of H, halo,



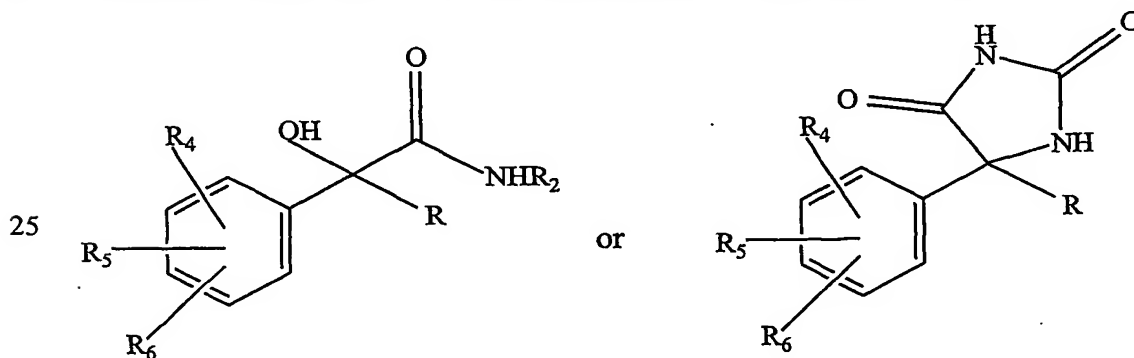
wherein R₁₁ is selected from the group consisting of H, C₁-C₄ alkyl, NH₂ and OH. In
30 one preferred embodiment the patient is treated with a compound represented by the general structure of Formula III or IV wherein R is selected from the group consisting of C₁-C₁₂ alkyl, R₂ is H, R₄ and R₅ are independently selected from the group consisting of H, halo and C₁-C₄ alkyl and R₆ is selected from the group consisting of H, halo,



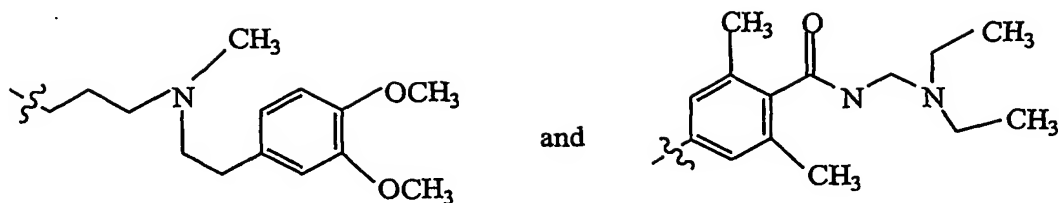
5 wherein n is 1 or 2. These sodium channel blocking compounds can be combined, or used in conjunction with, other known anti-tumor agents or therapies, such as chemotherapeutics or radiation treatments, to effectively treat cancer patients.

In accordance with one embodiment of the present invention a method for inhibiting voltage-gated sodium channel activity in a subject is provided, as a means of
 10 treating an illness associated with inappropriate sodium channel activity. The inappropriate activity will typically constitute channel hyperactivity or it may represent the expression of a voltage channel variant in a cell/tissue that normally does not express that channel. Existing sodium channel blockers have been used to treat a number of diseases, including epilepsy, bipolar disease, depression, pain, ALS, and arrhythmia. It is anticipated that the sodium
 15 channel blockers of the present invention will have utility as neuroprotective agents (including preventing secondary neuronal death after an initial injury) as well treating any of the disease states previously treated with sodium channel blockers.

In accordance with one embodiment a method of treating a disease state characterized by inappropriate sodium channel activity comprises the steps of administering a
 20 composition comprising a compound represented by the general structure:



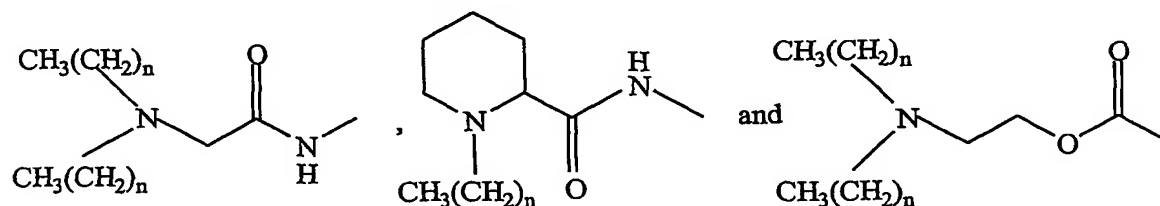
25 wherein R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, -(CH₂)_nC₃-C₆ cycloalkyl,
 30



R_2 is selected from the group consisting of H, C₁-C₈ alkyl, $-(CH_2)_nCOOH$, $-(CH_2)_nNH_2$, $-(CH_2)_nNHCH_3$, and $-(CH_2)_nCONH_2$;

R_4 and R_5 are independently selected from the group consisting of H, halo, C₁-C₄ alkyl, C₂-C₄ alkenyl, C₂-C₄ alkynyl, $-COR_{11}$ and (C₁-C₄) alkoxy; and

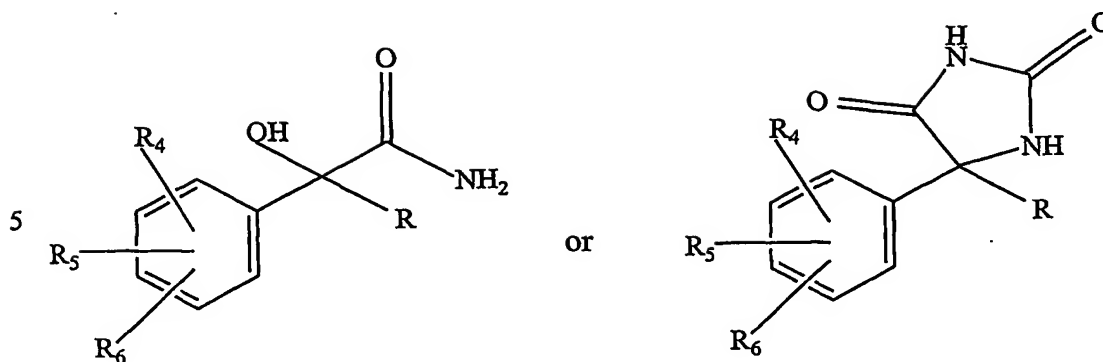
R_6 is selected from the group consisting of H, halo,



wherein n is an integer ranging from 0-4, and R_{11} is selected from the group consisting of H, C₁-C₄ alkyl, NH_2 and OH.

As disclosed herein two classes of sodium channel blockers have been demonstrated as being effective inhibitors of prostate cancer cell proliferation. Both hydroxyamides of the general Formula III and hydantoin of the general Formula IV were shown to inhibit androgen dependent and independent cell lines *in vitro*. Tritiated thymidine uptake assays in PC-3 cells using hydantoin analogue 44 showed 55% inhibition of DNA synthesis at 40 μM (see Table 3 in Example 4). Further, these analogues demonstrated only marginal impact on cell viability after 24 hours treatment (Table 4 in Example 4). The sodium channel blockers of the present invention also demonstrated cell selective inhibition of growth in a long-term growth assay over several cell lines. In development of prostatic neoplasia, the time from tumor initiation and progression to invasive carcinoma often begins in men in the fourth and fifth decades of life and extends across many decades. Because of the protracted course of this disease the use of chemopreventive strategies or cytostatic strategies may be ideal in the treatment of prostate cancer.

In accordance with one embodiment of the present invention a method is provided for inhibiting the proliferation of neoplastic cells, and more particularly in one embodiment, prostate cancer cells. The method comprises contacting the cells with a compound represented by the general structure:

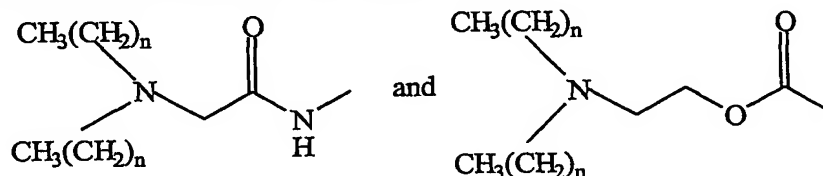


10 wherein R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl and C₂-C₈ alkynyl;

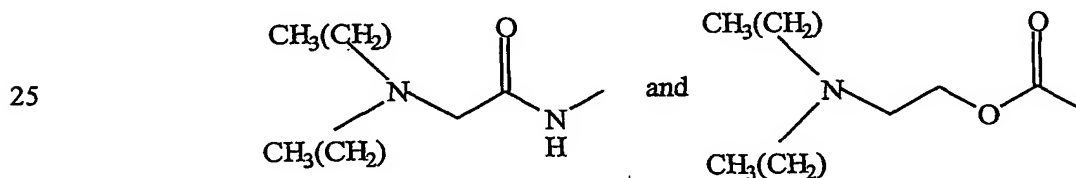
R₄ and R₅ are independently selected from the group consisting of H, halo, C₁-C₄ alkyl,

-COR₁₁ and (C₁-C₄) alkoxy; and

15 R₆ is selected from the group consisting of H, halo,



20 wherein R₁₁ is selected from the group consisting of H, C₁-C₄ alkyl, NH₂ and OH. In one embodiment R is selected from the group consisting of C₁-C₁₂ alkyl, R₄ and R₅ are independently selected from the group consisting of H, halo and C₁-C₄ alkyl, and R₆ is selected from the group consisting of H,



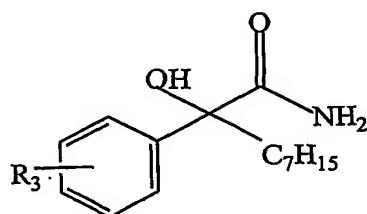
30 In another embodiment R is selected from the group consisting of C₁-C₁₂ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, R₄ and R₅ are independently selected from the group consisting of H and halo, and R₆ is H. These compounds can be further combined with pharmaceutically acceptable carriers and other therapeutic compounds (such as anti-tumor agents) to provide therapeutic pharmaceutical compositions for treating neoplastic diseases, including breast, glioma and prostate cancers.

The sodium channel blocker compositions of the present invention can be administered either orally or parenterally. In one embodiment the composition is administered locally by injection or by an implantable time release device. When administered orally, the compounds can be administered as a liquid solution, powder, tablet, capsule or lozenge. The compounds can be used in combination with one or more conventional pharmaceutical additives or excipients used in the preparation of tablets, capsules, lozenges and other orally administrable forms. When administered parenterally, and more preferably by intravenous injection, the sodium channel blockers of the present invention can be admixed with saline solutions and/or conventional IV solutions.

One embodiment of the present invention is directed to pharmaceutical compositions comprising the compounds of the invention and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be selected from among the group consisting of excipients, disintegrating agents, binders and lubricating agents. The amount of the pharmaceutical agent suitable for administration will be in accordance with standard clinical practice. The amount of the pharmaceutical agent suitable for administration will be in accordance with standard clinical practice. In addition the pharmaceutical compositions can be further combined with known anti-tumor agents and used in conjunction with known anti-tumor therapies.

Example 1

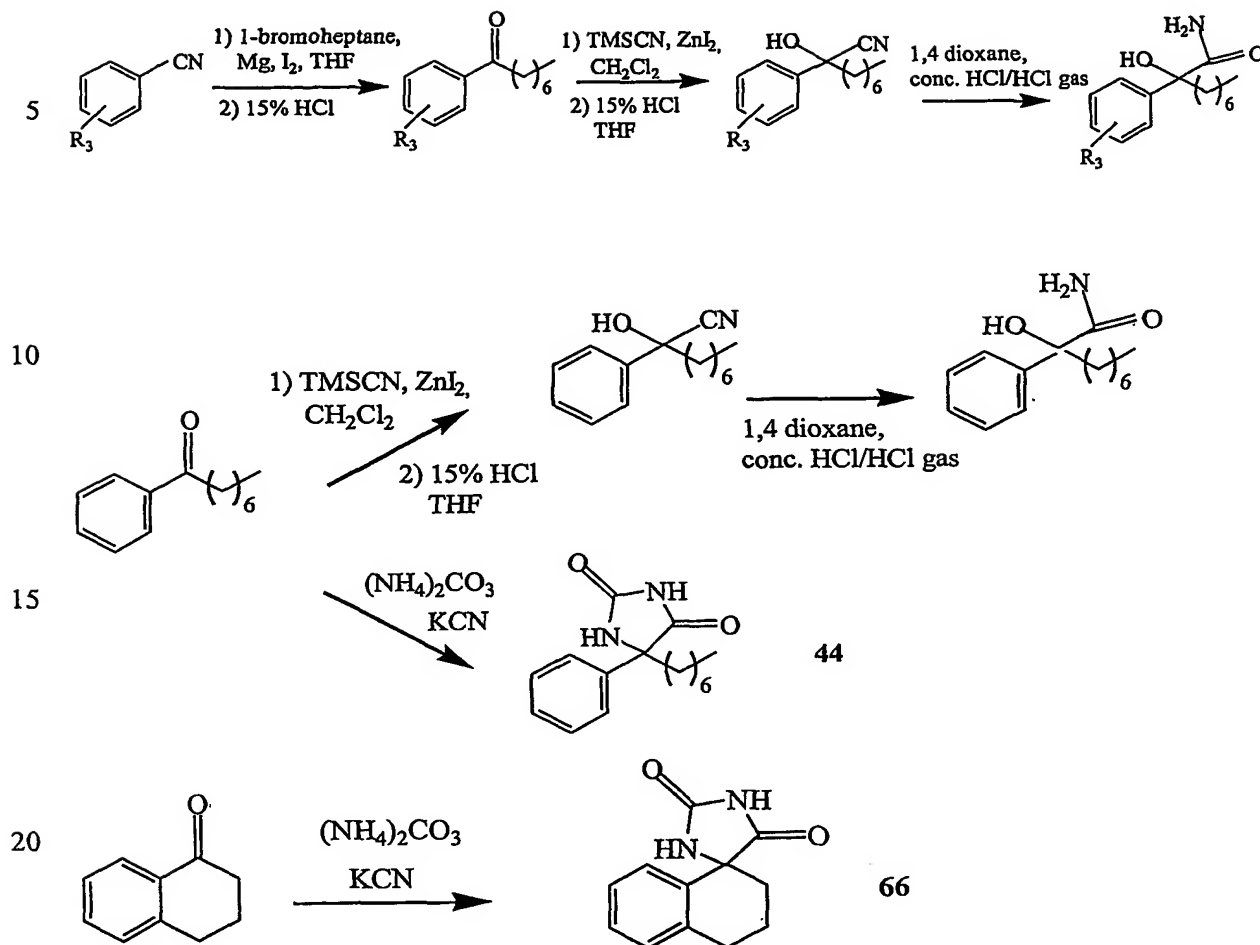
Organic Synthesis of the Proposed Compounds.



<u>Cmp</u>	<u>R₃</u>
1	3-Cl
2	4-Cl
3	2-Cl
4	4-OCH ₃
5	H
9	4-FI

Hydroxyamide compounds 1-5 were synthesized according to literature procedures and as outlined in Scheme I. In general, the corresponding nitrile was converted to the ketone by grignard addition followed by the conversion of the ketone to the TMS ether using TMSCN. The TMS ether was cleaved to the cyanohydrin with 1% HCl and the corresponding cyanohydrin hydrolyzed to the final product using concentrated HCl/HCl gas to generate the final compounds. Hydantoin analogues 44 and 66 were prepared by the Bucherer-Berg reaction from commercially available ketones.

Scheme I



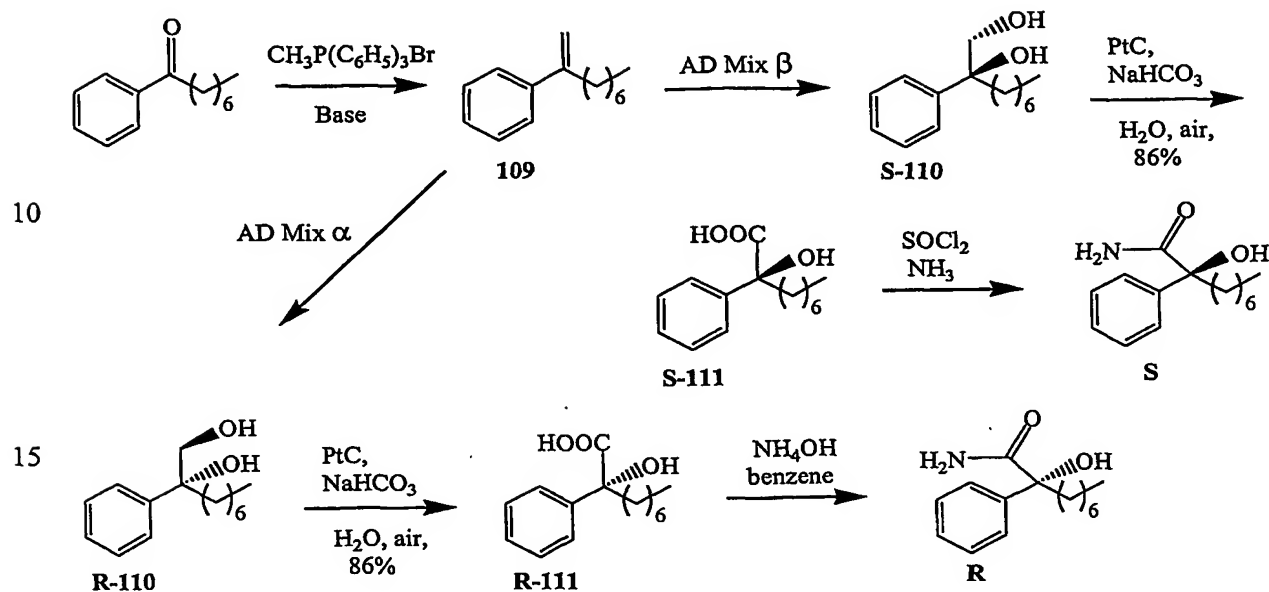
Enantioselective synthesis of hydroxyamides using a Sharpless dihydroxylation strategy.

25 The majority of the present compounds are chiral, thus it is anticipated that scheme for preparing multi-gram amounts of each enantiomer of active analogs will be desirable. With that in mind, a general synthetic scheme (Scheme II) was prepared and S(-)-2 was successfully synthesized. Using an enantioselective Sharpless dihydroxylation strategy, the alkene 109 was converted to the diol 110 in the presence of AD mix α or β . The chiral

30 diol 110 was oxidized to the chiral hydroxyacid 111 and converted to the chiral enantiomer with retention of stereochemistry. It is anticipated that this methodology can be adapted to synthesize any of the other enantiomers of racemic hydroxyamides proposed in this study that

prove to have effectiveness against prostate cancer cell proliferation and sodium channel activity.

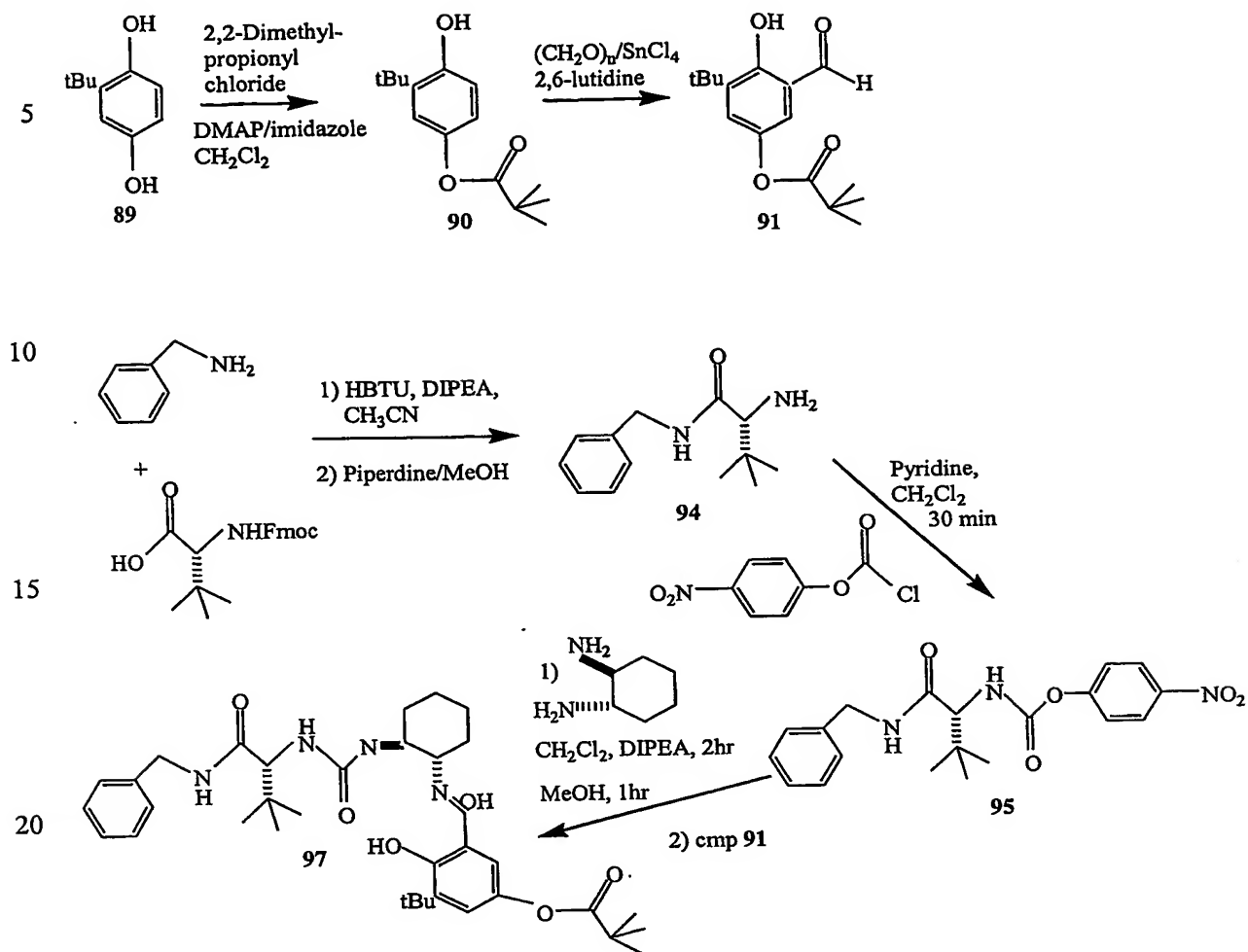
Scheme II



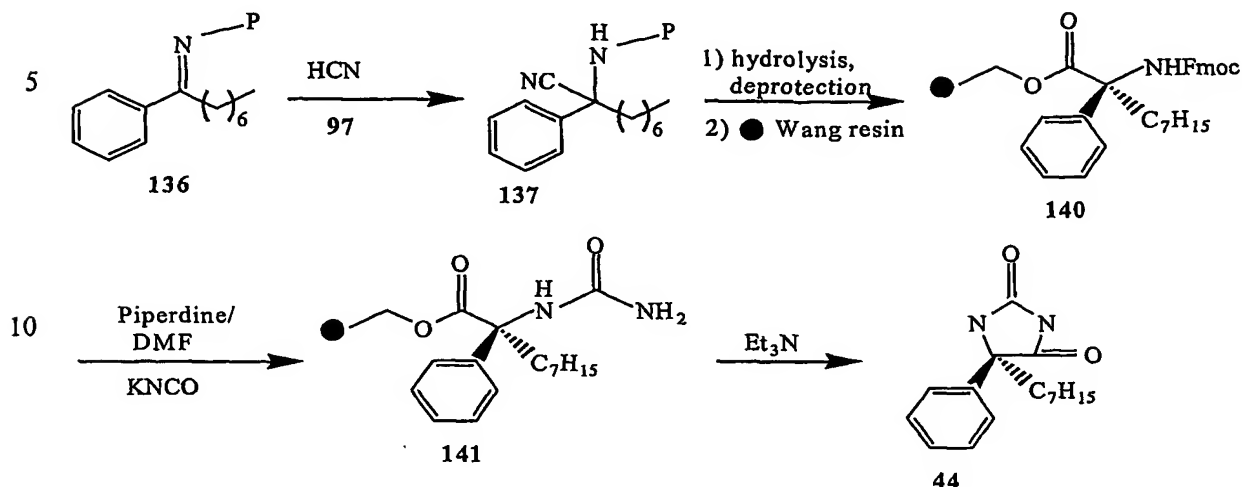
Enantioselective synthesis of the Hydantoin analogues using a Jacobsen Catalyst strategy.

The majority of the hydantoin compounds are also chiral, thus it is anticipated that scheme for preparing multi-gram amounts of each enantiomer of active analogs will be desirable. With that in mind, a general synthetic scheme (Scheme III) was prepared for synthesizing R(+)-44. Jacobsen's catalyst **97** will be used to synthesize the chiral hydantoins using the imine **136** as the starting material. A synthetic route for preparing Jacobsen's catalyst and the use of that catalyst to prepare chiral hydantoin analogs is shown in Scheme III. It is anticipated that this methodology can be adapted to synthesize any of the other enantiomers of racemic hydantoins proposed in this study that prove to have effectiveness against prostate cancer cell proliferation and sodium channel activity.

Scheme III



Jacobsen's catalyst **97** is then used to synthesize the chiral hydantoins (such as cmp **44**). The starting material will be the imine **136**:



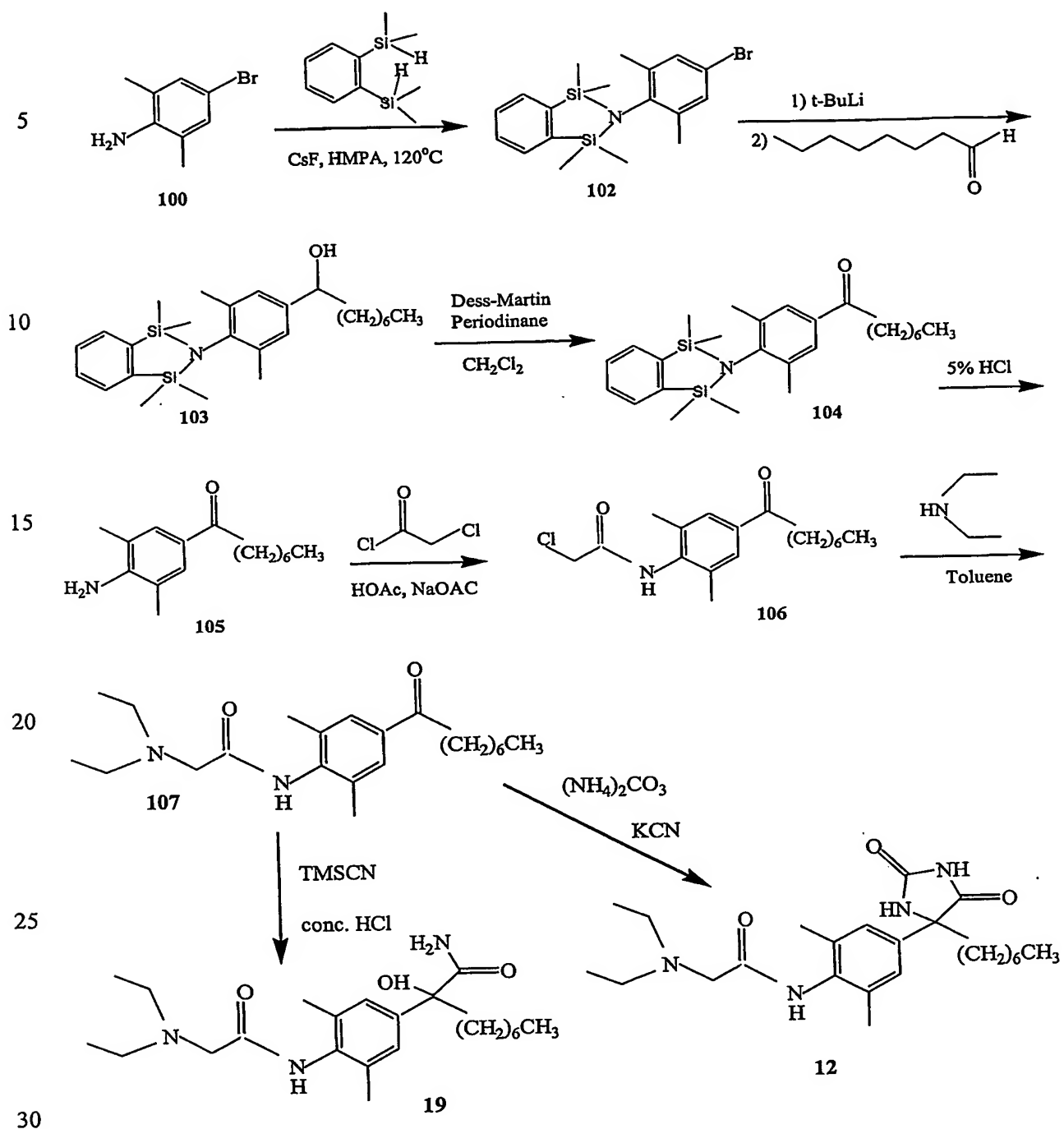
- 15 HCN will be added enantioselectively to the imine **136** in the presence of Jacobsen's catalyst (**97**) followed by hydrolysis and deprotection of **137** to give the chiral amino acid **138**. Protection of the chiral amino acid **138** will generate the Fmoc protected amino acid **139**. Compound **139** will be loaded onto Wang resin to give **140**. Deprotection with piperidine in DMF and addition of KCNO will generate the ureide **141**. Release from the resin and ring closure will be facilitated by reaction with Et₃N to form (+) or (-)-**44**.
- 20

Additional compounds will be prepared in accordance with the following schemes:

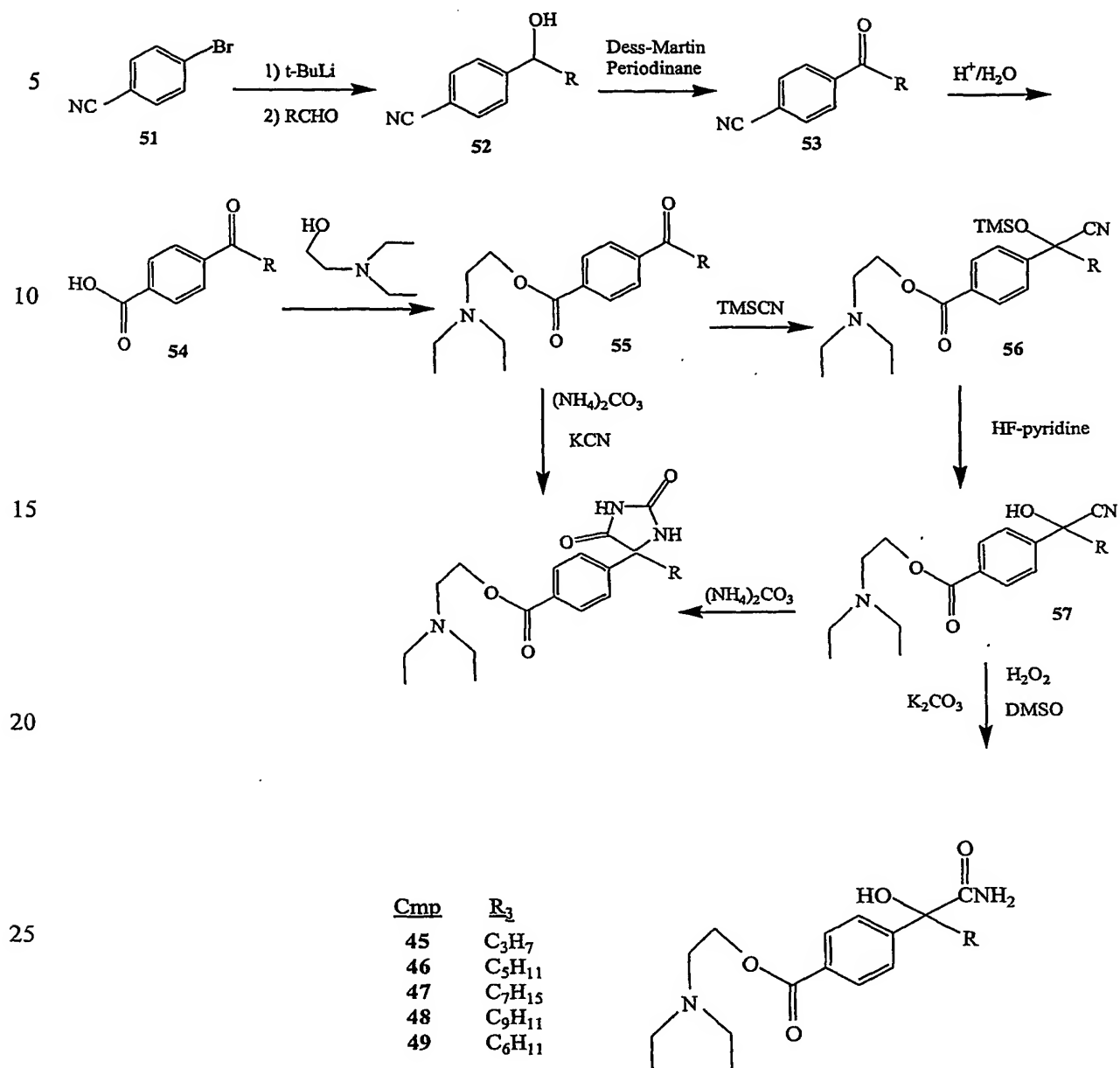
25

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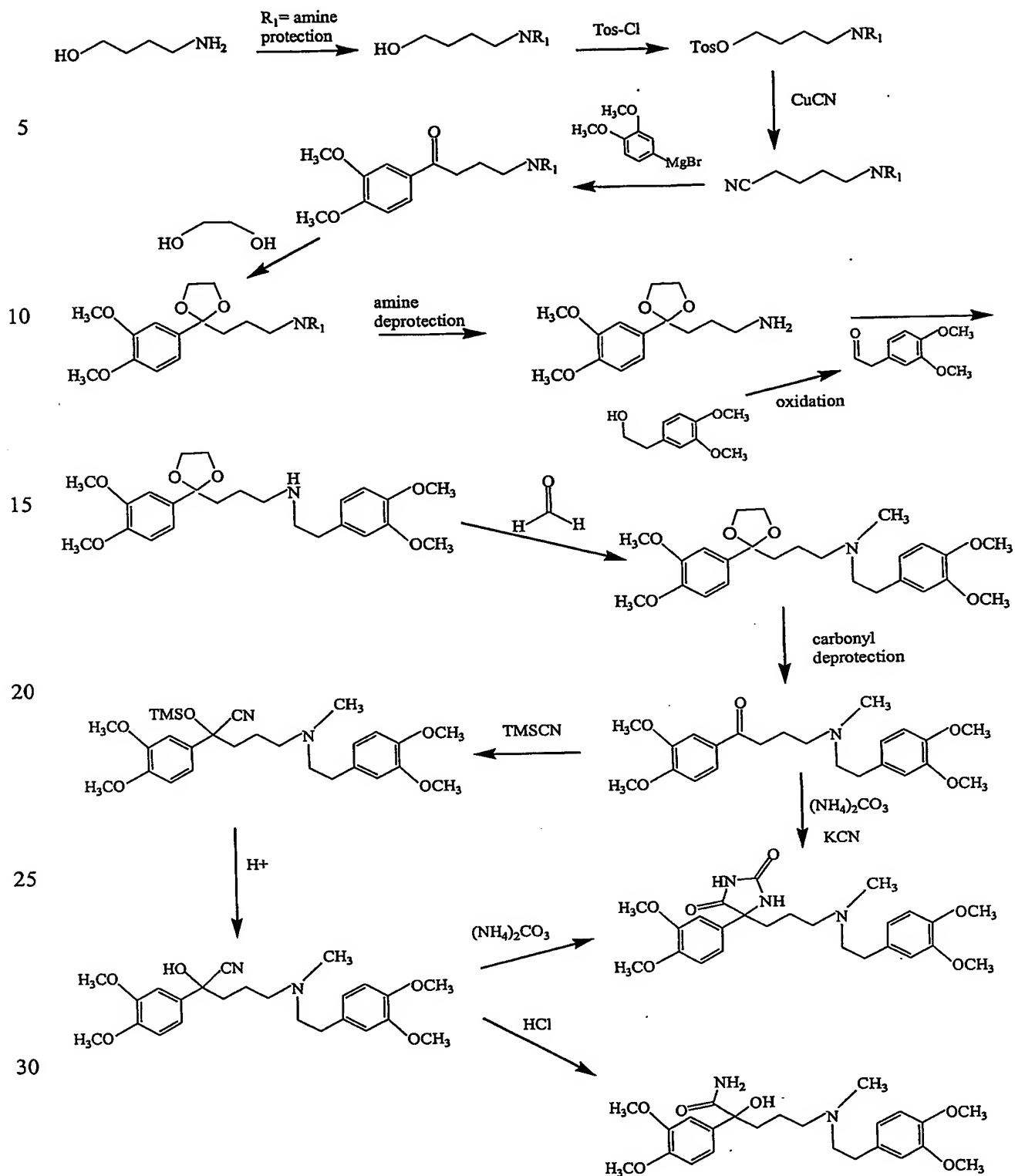
Scheme IV



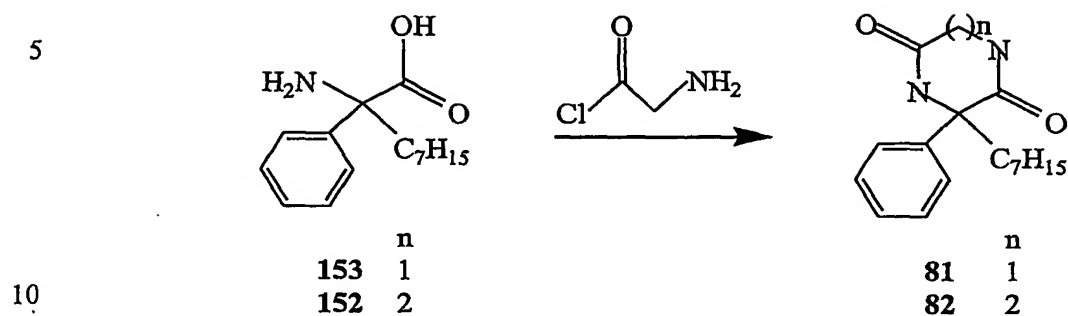
Scheme V



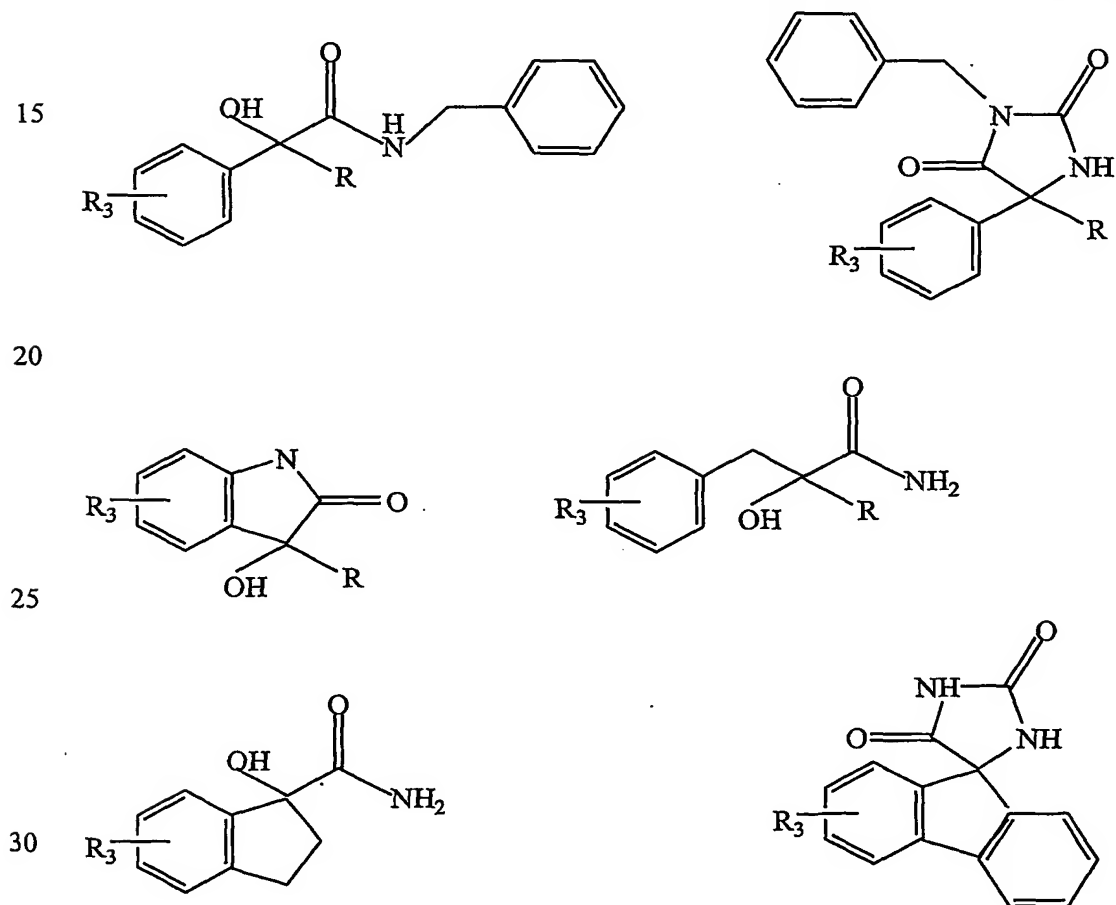
Scheme VI



Scheme VII



Additional compounds suitable for use in the present invention include the following:



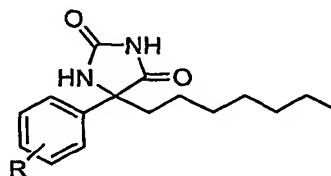
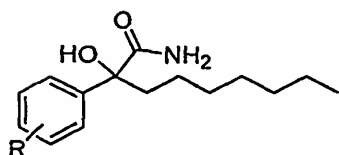
wherein R_3 is H or halo and R is $\text{C}_1\text{-C}_9$ alkyl.

Example 2**Effects of the Synthesized Compounds on 3H-BTX-B Binding**

One assay used to screen compounds for modulators of sodium channel activity, is based on the use of the radioligand 3[H]-BTX-B assay. BTX binds to site 2 on the channel protein and thus compounds that can compete with or inhibit BTX binding to the sodium channel are potential sodium channel inhibitors. This assay represents a facile tool to prescreen sodium channel binding before evaluating compounds in more rigorous functional assays such as through electrophysiology. Compound 2 and 44 demonstrate effective inhibition of 3H-BTX-B binding in comparison to phenytoin (see Table 1).

Table 1 3H-BTX-B Inhibition Data

Compound	3H-BTX-B (μ M)
5	9 ± 2
44	5 ± 1
phenytoin	40

 **α -hydroxyamides**

NAME	R	% BTX INHIBITION (40 μ M)
JDA-II-105	H	tbd
JDA-xx-xxx	2-Cl	tbd
JDA-III-145	3-Cl	46.14 ± 2.65
JDA-III-177	4-Cl	48.36 ± 1.18
JDA-IV-191	3,4-Cl	tbd
JDA-IV-069	2-F	37.88 ± 1.06
JDA-IV-111	3-F	47.96 ± 2.70
JDA-xx-xxx	4-F	tbd
SS-xx-xxx	2-OMe	tbd
JDA-IV-067	3-OMe	56.36 ± 4.33
JDA-III-271	4-OMe	52.55 ± 1.15
JDA-xx-xxx	2-Me	tbd
JDA-IV-093	3-Me	43.69 ± 0.51
JDA-IV-095	4-Me	25.37 ± 0.06

Hydantoins

NAME	R	% BTX INHIBITION (40 μ M)
JDA-I-073	H	tbd
JDA-III-105	2-Cl	41.61 \pm 5.36
JDA-III-135	3-Cl	69.57 \pm 3.34
SS-xx-xxx	4-Cl	tbd
JDA-III-113	3,4-Cl	9.25 \pm 3.25
JDA-xx-xxx	2-F	tbd
JDA-xx-xxx	3-F	tbd
JDA-III-179	4-F	11.71 \pm 4.51
JDA-II-053	2-OMe	35.29 \pm 0.46
JDA-II-047	3-OMe	37.80 \pm 0.64
JDA-IV-273	4-OMe	tbd
JDA-xx-xxx	2-Me	tbd
JDA-xx-xxx	3-Me	tbd
JDA-xx-xxx	4-Me	tbd

Example 3

15 Electrophysiology: Effects of the Synthesized Compounds on Sodium Currents.

The functional sodium channel blocking ability of several synthetic compounds was measured using electrophysiological. Using chinese hamster ovary cells (CHO cells) stably expressing the sodium channel isoform, Nav1.5, sodium currents were elicited from a holding potential of -120mV to a series of voltages ranging from -80mV to $+60\text{mV}$ in steps of 5mV for 25ms . These current-voltage recordings were made in the absence and presence of drug and following washout. All effects were fully reversible on washout thus indicating that the compounds were not toxic to the cells. Table 2 lists the recorded IC_{50} obtained for all the compounds tested. The results of these studies demonstrate directly that the present compounds block Na channel currents.

Table 2

Compound	EC_{50} (μM) n=5-7
1	14.7 \pm 0.4
2	12.7 \pm 2.7
5	166.4 \pm 44.1
9	34.2 \pm 7.4
44	29.8 \pm 4.2
phenytoin	>200

Example 4**Effects of Sodium Channel Blockers on Androgen Dependent, and Independent Cell lines.**

The novel sodium channel blockers were assayed for activity against paired sets of cell lines with increasing metastatic potential, PC-3 and PC-3M (already androgen independent, AI), or increasing metastatic potential with increasing androgen independence, LNCaP and C4-2. DU145 was also included to provide another AI cell line with intermediate tumorigenicity between the LNCaP series and the PC-3 derivatives. This strategy allows an examination of at least three different genotypes for comparison of drug efficacy. Several biological assays have been used in the initial screening of these sodium channel analogues. The first assay used to screen these compounds was ³H-thymidine uptake in PC-3 cells. The cells were trypsinized, equal cell numbers were placed into each well and allowed to recover overnight. The medium was changed the next day and the analogues were added at the concentration indicated in Table 3. Analogue 2, and the hydantoin analogue 44, inhibited DNA synthesis better than phenytoin at all dosages tested. Indeed, at higher concentrations, phenytoin was stimulatory as was analogue 4, at all dosages tested. These data confirmed the anticipated success of the design strategy that was invoked by the receptor targeted scheme outlined above.

Table 3**Inhibition of PC3 Cells**

	Concentration(μ M)						Compound
	100	40	20	10	1	0	
%Inhibition	30.75	-12.54	-21.78	-13.93	-23.77	0	Phenytoin
	81.99	55.09	32.70	26.64	12.75	0	44
	-13.36	-42.58	-71.62	-53.19	-41.03	0	4
	96.20	48.90	44.58	25.25	-3.61	0	2

To determine the novel sodium channel blockers are cytostatic or cytotoxic inhibitors, studies were performed using MTT after treating PC-3 cells with the analogues. In these assays the MTT compound is taken up by live cells in the culture and converted to insoluble formazan crystals by functional respiratoring mitochondria. These crystals can then be solubilized in DMSO or acid-EtOH and the absorbance measured at 570 nM to determine the relative number of viable cells in a culture. The results from these studies indicate that the compounds have only a marginal impact on cell viability after 24 hours of treatment. A

reduction in viability is only seen at the highest dose tested, 100 μ M. This dose is in great excess over the apparent IC₅₀ of 30 μ M for the inhibitory compounds.

Long-term growth assays (7 days with drug) were also initiated to determine if the effects observed were stable or transient. C4-2 cells were plated at cell densities established previously to reach saturation at day 7. The cells were allowed to recover overnight before a media change with the analogues (all at 40 μ M) was performed. The assays were terminated at day 1, 3, 5 and 7. Media changes were performed on day 0, 2, 4 and 6 with fresh analogues added each time. The cells were then fixed with glutaraldehyde and stained with crystal violet. The dye is then eluted in Sorenson's solution and the absorbance read at 540 nM. The results of a representative growth assay for C4-2 cells is shown in figure 10. These results show a remarkable inhibition of growth by the hydroxyamide compound 1. Hydantoin analogue 5, had intermediate efficacy while a similar analogue 66, had no discernible effect from the control and may have been mildly stimulatory as was also observed for the lidocaine control. These results with the AI, human prostate cancer cell line, C4-2, were not exclusive to C4-2. These assays were repeated for DU145, PC-3 and the paired metastatic cell line PC-3M. The data were normalized to the day 7 DMSO control for each cell line to allow for direct cell:cell comparison. These data demonstrated two important points. Firstly, the hydroxyamide analogue 1 quite effectively inhibited growth of all PCa cell lines tested to date inhibiting the cells' growth to a maximum of 20-25% of controls. Secondly, the hydantoin class of analogues, 44 and 66 show cell selective inhibition of growth. The IC₅₀ was determined for compounds 1, 2, 5 and 44 at day five for several of our channel blockers against the matched panel of PCa cell lines (Table 4). Remarkably, the data shows that compounds which demonstrated the most active sodium channel blockade (compounds 1 and 2 vs 5 and 44) were the best inhibitors of prostate cancer cell proliferation.

TABLE 4 Effects of Sodium Channel Blockers on Prostate Cancer Cell Proliferation (Day 5)

Day Five Effects on Cell line IC ₅₀ (μ M) \pm S.E.M.						Na ⁺ Channel Blockade
Compound	C4-2	PC3	DU-145	PC-3M	LNCaP	EC ⁵⁰ (μ M)
1	51.3 \pm 0.2	66.0 \pm 0.7	56.0 \pm 0.7	61.9 \pm 0.1	72.2 \pm 0.1	14.7 \pm 0.4
5	61.8 \pm 0.0	>100	84.3 \pm 2.2	69.9 \pm 1.3	81.3 \pm 0.5	166.4 \pm 44.1
2	61.1 \pm 0.1	66.8 \pm 1.2	67.2 \pm 0.1	50.0 \pm 0.0	64.1 \pm 4.3	12.7 \pm 2.7
44	67.4 \pm 1.3	66.0 \pm 0.1	35.6 \pm 2.6	58.4 \pm 0.0	71.8 \pm 1.8	29.8 \pm 4.2

Example 5**Effects of Sodium Channel Blockade on PSA Secretion and Cell Migration.**

PSA levels are clinically important in following tumor progression and burden. Sodium channel blockers 1, 2 and 44 (at 40 μ M) and phenytoin at (100 μ M) were investigated for their ability to suppress PSA secretion by PCa C4-2 cells. Compounds 1 and 2 significantly decreased PSA secretion on a per cell basis by 42.5% and 41.8%, respectively. Phenytoin was less effective at inhibiting PSA secretion (17.8% at 100 μ M) and compound 44 inhibited secretion by 14.9% at 40 μ M. Remarkably, this trend reflects the ability to block sodium channels and the effects on cell proliferation. Further, the dose-response curve of compound 1 for reducing PSA revealed an IC₅₀ of approximately 10 μ M, which matches the sodium channel EC₅₀ of 14.7 μ M. This raises very new and interesting questions as to the role of ion channels on PSA secretion pathways.

Soft agarose colony formation (SACF) is a technique for growing cells suspended in a 3-dimensional (3-D), semi-solid medium. Growing single cells in this manner using agarose deprives them of a substratum with which to adhere. One hallmark of cancer cells is their ability to grow in an anchorage-independent manner. Colony formation on soft-agarose simulates the 3-D, anchorage-independent growth of a tumor. When normal cells are grown under similar conditions they rapidly apoptose. In order to determine the possible efficacy of these compounds to inhibit tumor formation, several compounds were screened using SACF. The sodium channel blockers have increased inhibitory activity in 3-D growth relative to that seen in 2-D growth assays when tested using molar equivalence to the phenytoin IC₅₀. Compounds 1 and 2, which were the best compounds in 2-D growth assays (66.3% and 42.3% inhibition) increase to approximately 87.2% and 62% inhibition at the same concentration in 3-D growth as compared to phenytoin. It should also be emphasized that the colonies that do form are much smaller in the test compound plates as compared to phenytoin. Therefore, the reduction in colony formation is even more dramatic than indicated by the shear numbers alone. Secondly, compounds that had marginal impact on cell growth in 2-D, namely compound 2, show dramatically enhanced inhibition (41.5% of phenytoin) using SACF. These data indicate that several compounds have potential to inhibit tumor growth *in vivo*. Interestingly, phenytoin itself is somewhat inhibitory in these 3-D assays. Phenytoin inhibited 25.7% of SACF compared to the DMSO/EtOH control plates.

Evaluation of Compound 1 Toxicity in Mice.

In collaboration with the NIH National Institute of Neurological Disorders and Stroke, compound 1 has been evaluated for acute toxicity in mice (Table 5). The data reveals that compound 1 is tolerated up to 300 mg/kg with 3/4 animals exhibiting a short term (0.5 hrs) impairment of balance at this high dose. There were no reports of death, spasms or respiratory distress with i.p. administration of compound 1.

In summary, thymidine incorporation, MTT, crystal violet assays, PSA, soft agar colony formation and patch clamping methodologies have been used to date and provide insightful information as to the activity of the claimed compounds. Further, preliminary toxicity data obtained in mice reveal compound 1 to be tolerated up to 300 mg/kg with a half an hour impairment in balance. No acute toxic effects (death, seizures, ataxia, cardiac arrest or loss of respiratory drive) were observed. Taken together, these preliminary studies point towards two classes of compounds, hydroxyamide and hydantoin, which actively inhibit PCa cell growth.

TABLE 5

NIH Evaluation of Compound 1 in Mice for impairment of Balance on Rotorod

Dose (mg/kg) ^b	Time (hours)			
	0.25	0.5	1.0	4.0
30	-	0/4	-	0/2
100	0/4 ^a	1/8	1/8	10/4
300	-	3/4	-	0/2
^a Number of mice with impaired balance on the rotating rod/ total # of animals tested. No deaths, spasms or respirator distress reported. ^b Dose administered <i>i.p.</i>				